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COMPARISON OF STIRRED AND IMMOBILIZED CELL REACTORS FOR ETHANOL PRODUCTION

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Abstract

Biomass can be converted to sugars by hydrolysis with enzymes or mineral acids. These sugars can be converted into a number of chemical intermediates in biological reactors. Biological reactions are generally slow and selection of the most efficient reactor is important in these applications.

Immobilized cell reactors allow high cell densities and high throughput by attaching microorganisms to a fixed support. This paper compares the rate of production of ethanol from glucose by Saccharomyces Cerevisiae in a packed column and a stirred reactor.

Continuous stirred reactor studies showed a washout rate of .27 hr^{-1}. The optimum rate of alcohol production of 1.75 g/l-hr occurred at a dilution rate of .182 hr^{-1}.

In a 36" immobilized cell reactor, rates were found to be 7.4 g/l-hr, or about 4.2 times better than the stirred reactor. Sustained periods of operation of this type column are possible by removal of cell overgrowth with a gas purge. Immobilized cell reactors should also be more stable and should require lower power input than the mixed reactor.

1. INTRODUCTION

As world reserves of petroleum are depleted, new sources of carbon and hydrogen must be found to supply our chemical and energy needs. Large quantities of biomass are available in most parts of the world and could be used as an energy mechanism or as raw material for chemicals manufacture.

For example, the United States has unused agricultural residues from production of food. Recent studies indicate that these residues total 300 million tons per year (Anderson, 1972; Steffgen, 1973; Roller, et al., 1975; Green, 1975; Wilson and Freeman, 1976; Benson, 1977). Table 1 shows the availability of the common residues by type. Corn stover represents about half the total and are three times more abundant than any other residue. Moreover, the central states produce 70 percent of all corn (Sitton and Gaddy, 1975), so that collection and transportation could be centralized. Corn residue yields are high, .6 to 3.5 tons per acre, as shown in Table 1.

Table 2 lists the chemical composition of cellulosic residues. The primary constituents are pentosans, hexosans and lignin. By weight, corn stover is 15 percent pentosan, 35 percent hexosan and 15 percent lignin. The pentosan and hexosan fractions (hemicellulose and cellulose) can be converted into energy or chemicals. Conversion methods include direct combustion, pyrolysis or biological conversion. Bioconversion is preferred because of higher efficiencies and preservation of minerals and nutrients for return to the soil.

Bioconversion of cellulose and hemi-cellulose requires hydrolysis of the polymers to monomeric sugars (hexoses and pentoses). Hydrolysis is catalyzed by

Table 1. Quantities of Agricultural Residues in the United States (millions of tons/yr)

Crop	Green (1975)	MRI (1977)	Yield (tons/acre)
Corn	143	156	0.6 - 3.5
Wheat	47	49	0.3 - 2.5
Soybeans	46	40	0.5 - 1.1
Sorghum	27	24	0.6 - 2.4
Oats	14	12	0.4 - 1.3
Barley	-	-	0.4 - 1.9
Rice Straw	-	-	1.8 - 2.8
Peanut Hay	-	-	0.2 - 1.6
Total	277	299	

Table 2. Chemical Analyses of Agricultural Residues and Wood

Material	Percent Pentosan	Percent Hexosan	Percent Lignin
Corn Stover	15.0	35.0	15.0
Corn Cobs	28.1	36.5	10.4
Wheat Straw	19.0	39.0	14.0
Rice Straw	17.0	39.0	10.0
Oat Hulls	29.5	33.7	13.5
Bagasse	20.4	41.3	19.9
Pine	7.4	52.3	26.6
Oak	19.6	44.8	24.8

enzymes from microorganisms or by mineral acids. Using dilute and concentrated sulfuric acid, in two stages, has yielded 95 percent conversion of pentosans to xylose and 90 percent conversion of hexosans to glucose in the University of Missouri laboratories. The sugars can be converted into alcohols, acids, aldehydes or gases by a number of biological pathways. The microorganism selected and the environmental conditions determine the products obtained.

A ton of corn residue produces 290 pounds of ethyl alcohol, based upon 90 percent conversion of the hexosan to glucose and 90 percent conversion of glucose to alcohol. To meet the annual U.S. requirement of ethanol (2 billion pounds (U.S. Trade Commission Reports, 1977)) would require only five percent of the available corn residues. Furthermore, the potential revenue is $51.90 per ton of residue, based on the present price of $.17 per pound of 95 percent ethanol (U.S. Trade Commission Reports, 1977). Therefore, biomass could supply a substantial quantity of chemical intermediates and the economic potential appears sufficient to justify commercialization.

2. BIOREACTORS FOR CONVERSION OF SUGARS INTO CHEMICALS

Biological reactions are slow by comparison with most chemical reactions; therefore, large fermentors would be required to produce substantial quantities of chemical products. Many different types of reactors have been investigated for biological reactions.

Batch reactors have been studied for conversion of wood sugars into ethyl alcohol. Studies at Forest Products Laboratory indicate complete conversion requires three days (Harris, et al., 1946). The batch process is cyclic and the cell culture experiences all phases of the growth cycle. Much faster reaction times would be necessary if biomass conversion were employed on a large scale.

Continuous stirred reactors improve the apparent kinetics. These systems pump substrate solutions through the reactor continuously. Flow rate and reactor volume determine the retention time. The concentration of a component is the same everywhere in the reactor. Therefore, the rate that cells leave

the reactor and hence, the conversion, depends on the retention time. Moreover, if the retention time is short, the cell culture washes out of the reactor. Reactors that handle low substrate concentrations are operated near washout conditions to minimize reactor size; and flow stability is critical.

To increase the rate of conversion in continuous mixed reactors, cells can be recovered from the reactor effluent and recycled. A gravity separator is usually used to collect cells by settling. Wilke (Margaritis and Wilke, 1978) has used this type reactor with a centrifuge for cell recovery in converting glucose to ethanol with yeast. This study showed about a tenfold increase in rate with cell recycle. The cost of centrifugation is, of course, a disadvantage with this reactor arrangement. A common problem in these systems is inhibiting substances. Each cell senses the same concentration of these inhibiting materials in a stirred reactor. Toxic materials in the feed substrate or the end product itself may inhibit cellular metabolism and cellular growth rate, thereby decreasing conversion.

The cell washout problem can also be overcome by use of a filter in the reactor to separate cells from the effluent. Wilke (Margaritis and Wilke, 1978) has experimented with this type system, called a rotor-fermentor, and found about a 10 fold rate improvement over the stirred reactor. These studies were conducted with yeast utilizing 10 percent sugar solutions at 100 psi. The higher pressure of this system and the pressure losses across the filter will adversely affect the cost of this system.

Another system that has been used to increase the reaction rate is the fixed-film or immobilized cell reactor. In this reactor, the substrate is passed over a film of organisms, attached to a solid support (Griffith and Compere, 1975). The support holds the organisms in place allowing higher substrate flowrates. Moreover, there is a higher density of organisms in the film than in a suspended culture; and higher conversions are possible. Furthermore, the biological film is stable. Outer layers of cells in the film may buffer the inner layers against toxic materials. Fluctuations in flowrate cannot wash out the culture since the support holds the bacteria in place.

The purpose of this paper is to examine the reaction rate of conversion of glucose to ethanol by Saccharomyces Cerevisiae in an immobilized cell reactor. These rates are compared to rates for this same reaction in a continuous mixed reactor.

3. EQUIPMENT AND EXPERIMENTAL PROCEDURES

The organism used in this study was Saccharomyces Cerevisiae (ATCC 24858). Table 3 lists the media employed in both the mixed and fixed film reactors.

Table 3. Media Component Concentrations

Component	GMS/L
Glucose (Fisher ACS)	30
Yeast Extract (ICN)	8.5
Ammonium Chloride (Fisher ACS)	1.3
Magnesium Sulfate (Fisher ACS)	0.1
Calcium Chloride (Fisher ACS)	0.06
Antifoam (Sigma Chemical)	33 µℓ

A New Brunswick, Model C-30, bench-top chemostat was used for the stirred reactor studies. Operating volume of this chemostat was 340 ml. Ten milliliters of the culture was used as inoculum for the chemostat. After eight hours, flow was begun. Temperature was controlled at 25.0 ± 0.5°C and agitator speed was 200 rpm. pH was controlled at 4.0 by addition of sulfuric acid or sodium hydroxide.

The chemostat was operated at various dilution rates. Five retention periods were allowed between each dilution for steady state conditions to be achieved. At steady state, the effluent was sampled and analyzed for sugar, ethanol and cell concentrations. The data were collected when three consecutive samples analyzed the same at the .05 level of significance.

The fixed film reactor was constructed of two inch (inside diameter) plexiglass tube, forty inches long. Sample ports were installed on two inch centers along the reactor length. A perforated plate at the inlet to the column radially distributes the substrate solution. Treated ceramic Raschig rings (1/4 inch nominal size) are randomly packed to a bed depth of thirty six inches. Table 4 lists the column characteristics.

The fixed-film reactor, filled with treated Raschig rings, was sterilized with ethylene oxide, then filled with inoculating culture. After four hours, flow was started at a low rate and continued for four more hours, after which the flow was increased to the test rate. The column was operated at room temperature of 22°C and pH of the feed was adjusted to 4.0. Samples were

Table 4. Packed Column Characteristics

Packing	1/4 in. Raschig Rings
Bed Depth	36.0 inches
Treatment	25% Gelatin, Crosslinked
No. Pieces	5840
Packing Wt.	2043 gms
Total Reactor Volume	1853 mls
Total Void Volume	902 mls

taken from each of the ports and analyzed for sugar and ethanol concentrations every two days.

Glucose concentrations were measured by the DNS method. Ethyl alcohol was determined by gas-liquid chromatography using n-propanol as an internal standard. Cell density was measured optically.

4. RESULTS AND DISCUSSION

The cell, substrate and product concentrations from the stirred reactor studies are shown as a function of dilution rate in Figure 1. As expected, the cell and product concentrations decrease and the substrate concentration increases as the dilution is increased. This figure shows that the cell and product concentrations go to zero at a washout rate of about .27 hrs^{-1}. Such a declining cell population, caused by dilution, is typical of the mixed reactor performance; and, of course, represents a major limitation in autocatalytic or biological reactions. The rate of ethanol production is seen to go through a maximum of 1.75 gm/l-hr at the optimum dilution rate of .182 hrs^{-1}.

Figure 2 shows the concentration profile in the fixed-film reactor 48 hours after start up. The flow rate for this run was 250 mls/hr. The immobilized cell reactor converts all of the sugar into ethyl alcohol, yielding 15.3 g/l in the effluent. Since carbon dioxide gas is a major by-product of the reaction, gas bubbles occupy a percentage of the void space. For the data of Figure 2, the gas holdup was 383 mls, measured by draining the column, after sampling, to determine the liquid holdup. The retention time based on actual liquid in the column was 2.07 hours, corresponding to a dilution rate of .483 hrs^{-1}. This dilution is almost twice the washout rate and 2.5 times the optimum dilution for the mixed reactor. For the fixed film reactor, the apparent reaction rate is 7.4 gm/l-hr, based on liquid holdup. This rate is 4.2 times larger than the maximum rate with the stirred reactor.

A major disadvantage in the fixed-film reactor is growth of the film into the void volume. Within two weeks of operation at the condition of Figure 2, the liquid void volume decreased from 900 mls to 183 mls. Accordingly, liquid velocites increased and severe channeling resulted. Figure 3 shows the scatter in the data resulting from channeling. Also, the conversion decreased from 100 percent to 75 percent.

The improvement in rates obtained with the tubular reactor would be offset by this shortened life of the system. However, it was found that the column could be regenerated by passing a high flow rate of nitrogen gas through the packing for a period of a few minutes. The gas dislodges the film growth from the void volume. Figure 4 shows the concentration profile after one such regeneration. As noted, conversion returned to 100 percent and the rate is actually slightly better than for the new column performance, shown in Figure 2. Sustained operation at these levels of performance were obtained for a period of several months, by successive regeneration about every two weeks. Product carbon dioxide could be used for regeneration. Also, the cells obtained in this manner may be reclaimed as a by-product.

5. SUMMARY AND CONCLUSIONS

Continuous stirred reactor studies of the production of ethyl alcohol from glucose by Saccharomyces Cerevisiae showed a washout rate of .27 hr^{-1}. The optimum rate of alcohol production of 1.75 g/l-hr occurred at a dilution rate of .182 hr^{-1}.

In a 36" immobilized cell reactor, rates were found to be 7.4 g/l-hr, or about 4.2 times better than the stirred reactor. Sustained periods of operation of this type column are possible by removal of cell overgrowth with a gas purge. Immobilized cell reactors should also be more stable and should require lower power input than the mixed reactor.

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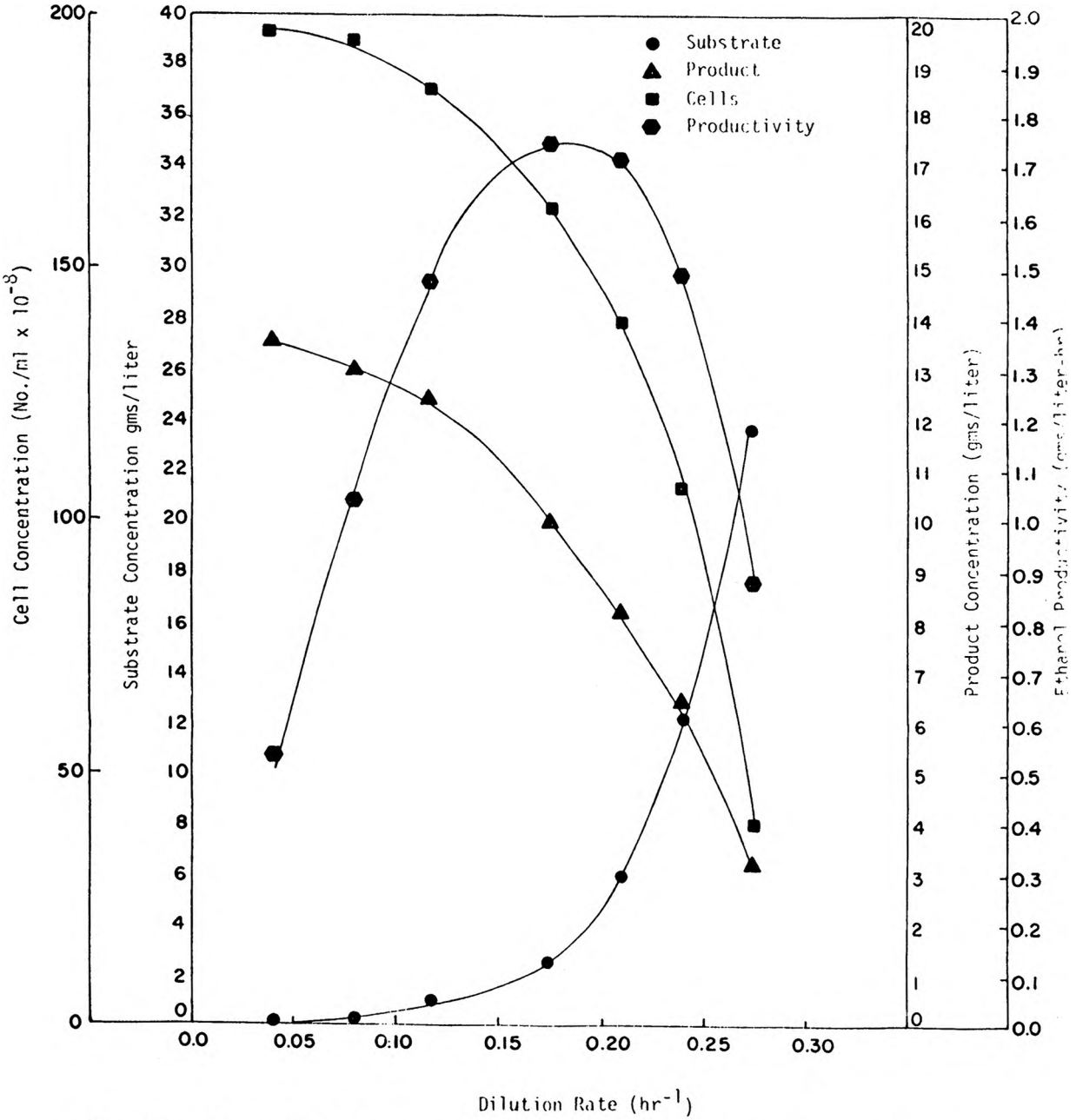

Figure 1. Chemostat Performance, S_0 = 30 gms/l, T = 25°C, Saccharomyces Cerevisiae

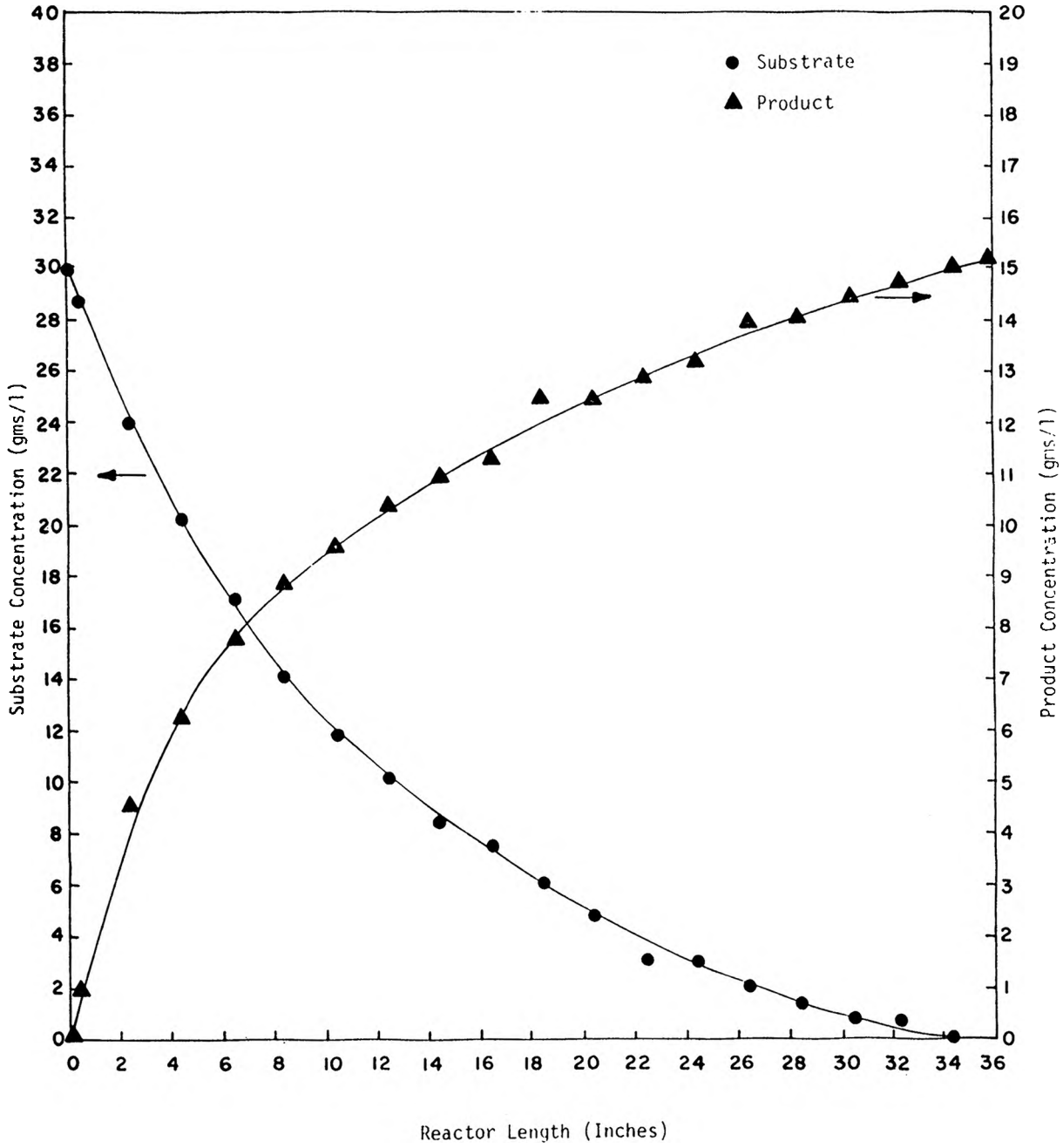

Figure 2. Performance of Immobilized Cell Reactor After One Day

Flow Rate = 250 mls/hr, S_0 = 30 gms/l, T = 22°C

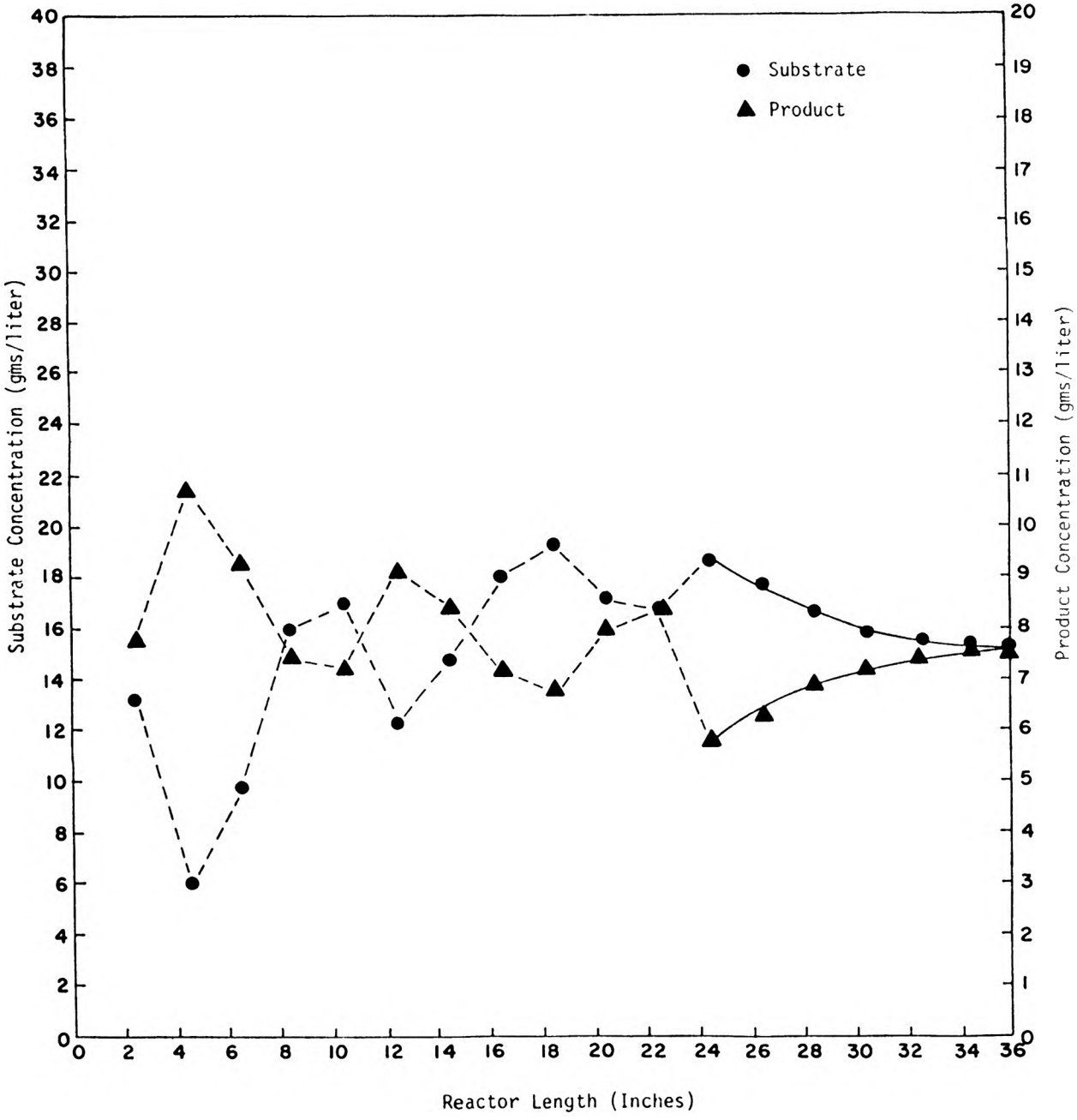

Figure 3. Performance of Immobilized Cell Reactor After 14 Days

Flow Rate = 250 mls/hr, S_0 = 30 gms/l, T = 22°C

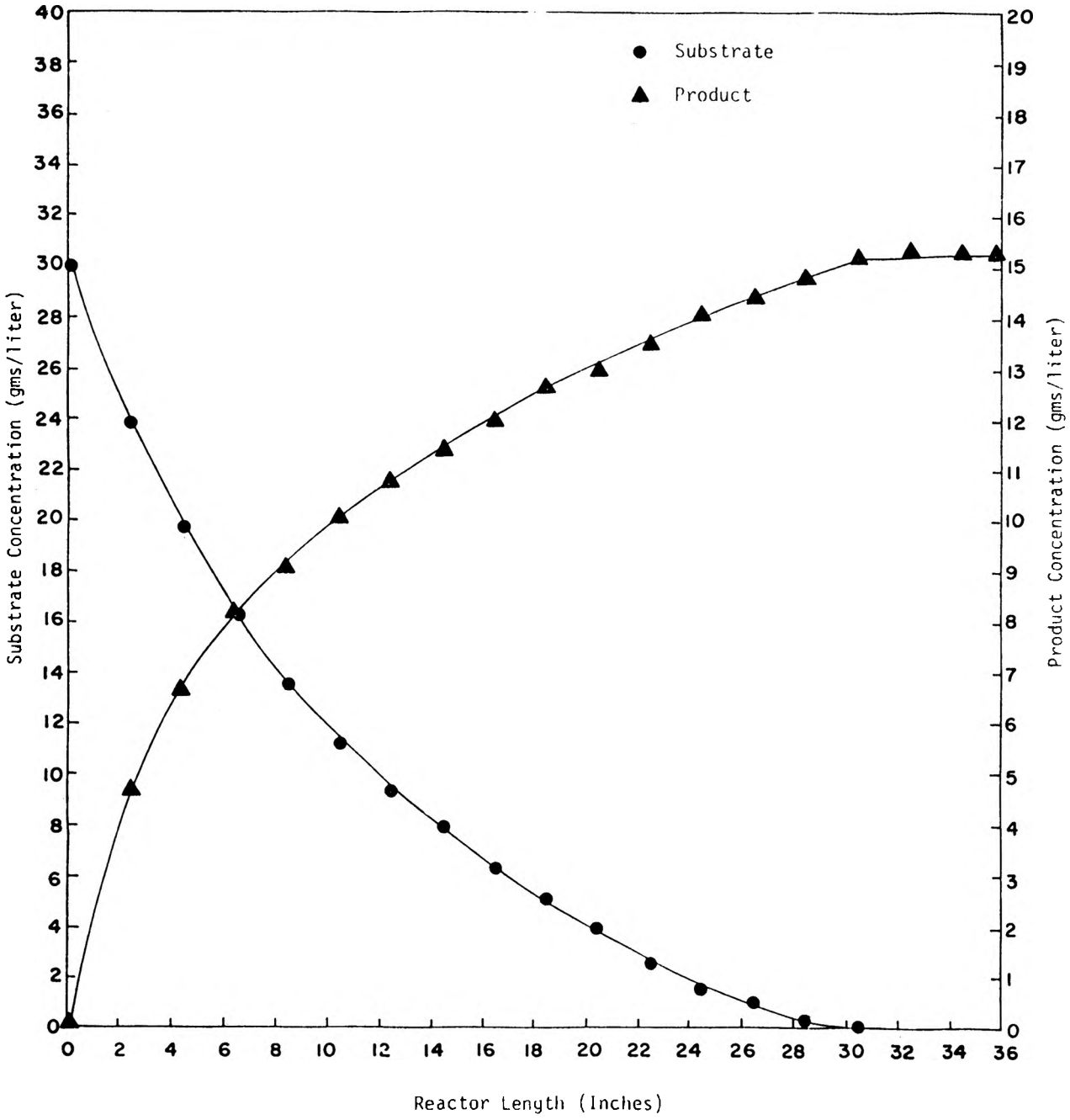

Figure 4. Performance of Immobilized Cell Reactor After Regeneration

Flow Rate = 250 mls/hr, S_0 = 30 gms/l, T = 22°C